(FILE 'HOME' ENTERED AT 16:29:38 ON 19 NOV 2002)

FILE 'MEDLINE, BIOSIS, CANCERLIT, LIFESCI, CAPLUS, BIOTECHDS' ENTERED AT 16:30:11 ON 19 NOV 2002

L1	165	S	SA(2W)(GAL OR GALACTOSIDASE)
L2	7385	S	(B OR BETA)(W)(GAL)
L3	44	S	L2(3A)(SENESC?)
L4	447	S	GALACTOSIDASE(3A)(SENESC?)
L5	540	S	L1 OR L3 OR L4
L6	251	S	L5 AND (TUMOR# OR TUMOUR# OR CANCER# OR CARCINOMA# OR ADENOC
L7	145	S	L6 AND PY<2001
L8	4	S	L5 AND CHEMOTHERA?
L9	0	S	L8 NOT L6
L10	48		DUP REM L7 (97 DUPLICATES REMOVED)
L11	1	S	L10 AND CHEMOTHERA?
L12	240	S	(BACUS,S?)/AU OR (BACUS S?)/AU OR (BACUS, S?)/AU
L13	1	S	L5 AND L12

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L19: Entry 1 of 3 File: USPT Mar 14, 2000

DOCUMENT-IDENTIFIER: US 6038041 A
TITLE: Three-dimensional holographic fluorescence microscope

Detailed Description Text (20):
Referring to FIG. 3, radio frequency signal generator 13 operating at 40 MHZ and radio frequency signal generator 14 operating at 5.35 MHZ are used to generate the drive signal for the AOM shown in FIG. 1. A frequency doubler 17 is used to obtain the heterodyned frequency, 10.7 MHz, for demodulation. To avoid excess loading of the signal generator, a signal splitter 15 is used between the first mixer 16 and the frequency doubler. The light levels involved in fluorescense microscopy are very low, so a PMT 10 must be used to collect the light. The signal out of the PMT, already amplified appreciably, must undergo additional amplification and filtering to detect the heterodyned signal. Two bandpass filters 20 and two amplifiers 21 are used in the electrical path between the PMT and the demodulation mixer 18. The filters have a 10.5 MHZ center frequency. One filter is placed in the signal path between the PMT and the first amplifier, while the other is placed after the second amplifier. The demodulated current, now at baseband, is filtered by a low pass filter 22 to eliminate high frequency noise. The A/D board 23 acquires the hologram by converting the analog voltage out of the low pass filter to a digital signal in sync with the scanning signals.

L19: Entry 2 of 3 File: USPT Apr 2, 1996

DOCUMENT-IDENTIFIER: US 5504337 A
TITLE: Method and apparatus for performing phase fluorescence lifetime measurements in flow cytometry

Brief Summary Text (28):
In one embodiment, the detecting step is performed by means of a PMT, and the output signal is generated as a ratio of the the split PMT signals. The PMT signal is split, one of the signals is phase shifted 90.degree., and the two signals are ratioed to create an intensity-independent amplitude which reveals the phase angle. In another embodiment the PMT signal is mixed with a variable-phase input signal, and the mixer output is integrated and used to control the input signal to provide to the integrator a voltage output that corresponds to the phase shift of the emission signal relative to the phase of the input signal.

Brief Summary Text (32):
According to another aspect of the present invention, there also is provided an apparatus for determining the lifetime of at least one fluorophore that is associated with a cell or particle in a flow cytometer, comprising a source of high intensity light at a selected wavelength capable of exciting the at least one fluorophore and modulated by an input signal at a set frequency, the modulated light being directed onto individual cells or particles, associated with the at least one fluorophore, passing an observation point in the flow cytometer to excite the fluorophore, a detector for detecting the emission from the excited fluorophore at the observation point to generate an emission signal, circuitry for splitting the modulating input signal into first and second signals that are in phase quadrature and for splitting the emission signal into third and fourth signals, circuits for mixing each of the first and second modulating signals with a respective one of the third and fourth emission signals, and ratio circuitry for creating the ratio of each of the two pairs of mixed signals to provide a lifetime signal which corresponds to the lifetime of the at least one fluorophore, such that the lifetime measurement is independent of the intensity of the fluorescence emission from the excited fluorophore.

Detailed Description Text (31):
The flow cytometer with phase fluorescence lifetime measurement ability, as seen in FIG. 2, also has an autofluorescence signal suppression capability. The light detection system may be adjusted electrically in such a way that the output signals of unwanted autofluorescence and/or non-rejected stray light become equal to zero. As seen in FIG. 2, the variable phase shifter 22, which receives the output of modulator driver 21 (or the detected output of a mode-locked laser) and provides that modulation signal to power splitter 23, may be set to provide a zero phase shift in a first embodiment. However, in a second embodiment, the signal contribution of unwanted autofluorescence can be suppressed if the variable phase shifter 22 is adjusted so that the phase difference for autofluorescence radiation at phase detector 26 is equal to zero. Alternatively, the phase of the LO input to 25 is adjusted to be 90.degree. shifted from the input signal to 25. By reducing the autofluorescence signal to zero, fluorescence with a lifetime different from the autofluorescence lifetime can be easily detected. When A is the phase shift angle resulting from the autofluorescence lifetime .tau..sub.A, the variable phase shifter 22 has to be adjusted to a shift angle equal to A. Under these conditions the output amplitude of the ratio device for autofluorescence present may be given by the expression:

Detailed Description Text (35):
In particular, in FIG. 6, the split output signal 24a of the power spitter 24 (FIG. 2) is fed via the high pass filter 202 to an additional non-phase shifting power splitter 201 which provides two split output signals 201a and 201b which are routed through

respective limiters 215a and 215b after amplification in respective variable gain amplifiers 213 and 214. The modulating signal from the frequency generator 21 and the phase-shifter 22 shown in FIG. 2 is also split by a power splitter 23 in two equal output signals 23a and 23b.

Detailed Description Text (36):
The split output signal 23b is phased shifted by 90 degrees in a phase shifter 28, while the other split output signal 23a is kept in phase with the output of the frequency generator 21 (FIG. 2). The in-phase and phase-shifted signals are amplified by respective amplifiers 219a and 219b, limited by respective limiters 220a and 220b and fed to respective double balanced mixers 203a and 203b where they are mixed with the split and limited fluorescence output signals 201a and 201b, respectively. The resulting output signals of the mixers 203a and 203b are low pass filtered via low pass filters 204a and 204b and peak detected via peak detectors 205a and 205b, which are gated by the comparator circuit 207 to produce two output voltages 206a and 206b corresponding to cosine and sine, respectively of the emission signal of the detector PMT 29 of FIG. 2. The gating is accomplished via the comparator 207 which comprises a preamplifiers 207a and a comparator 207b whose output signal gates or enables the peak detectors only after the envelope of each peak has passed a predetermined threshold value, determined by a reference signal inputted by the comparator 207b as a predetermined threshold level corresponding to amplitude limits encompassing the amplified and limited fluorescence output of the detector PMT 29 (FIG. 2), and disables the detectors 205a and 205b when the signal envelope drops below the threshold. The ratio of the output voltages 206a and 206b is then determined by a modulation ratio unit 221 whose output is intensity independent, since the peak voltage is representative of the tangent of the phase and/or modulation change between the fluorescence output signal originating from the detector PMT 29 and the reference signal from the frequency generator 22 (FIGS. 2). This change in phase and/or modulation corresponds to the fluorescence lifetime of the fluorophore associated with the cell or particle passing through the flow cell chamber 3 (FIG. 2). To determine this change in phase and/or modulation, the output voltage from the modulation ratio unit 221 is fed into the signal processor 30.

Detailed Description Text (40):
The fluorescence electrical output signal, after 10 dB of amplification by a preamplifier 103, is split into two equal signals 104a and 104b, respectively, by a power splitter 104 while maintaining fidelity and relative amplitude without inducing additional intensity dependent phase shifts. A first signal 104a from the power splitter 104 is fed through a high pass filter 105 and then mixed and further amplified in a variable gain amplifier 106, whose output 106a is split by a power splitter 112 to provide a first split signal 112a which limited to a suitable operating range by a limiting circuit 107. The second split signal 112b is peak detected in a peak detector 114c forming part of a gated peak detector circuit 114.

Detailed Description Text (50):
The signal 104b obtained from the power splitter 104 is filtered by a low pass filter 113 to produce a low pass filtered signal detected via a gated peak detector circuit 114 comprising a comparator 114a and a peak detectors 114b and 114c. The amplitude of the low pass filtered signal is gated to the peak centroid via the comparator with a reference signal, inputted as a predetermined threshold level corresponding to amplitude limits encompassing the amplified and limited fluorescence output of the detector PMT 102, and a peak detector 114b to produce an output signal B. The high pass filtered signal, originating from the power splitter 104 as signal 104a, is gated via a peak detector 114c to produce a signal A. The gated high pass filtered signal A is then compared to the gated low pass filtered signal B and a modulation ratio is determined via a modulation ratio unit 116.

CLAIMS:

6. A method of determining, in a flow cytometric environment, a fluorescence lifetime of at least one fluorophore associated with a cell or particle, comprising the steps of:

directing a stream of a plurality of said cells or particles past an observation point;

modulating a light source at a frequency of an input signal to produce modulated exciting light having a wavelength capable of exciting said at least one fluorophore;

irradiating at the observation point said cell or particle with said modulated light to produce emitted light from said at least one fluorophore;

detecting said emitted light and producing a corresponding modulated emission signal; and

comparing said input signal and said modulated emission signal to produce an output signal corresponding to a difference between the phase and/or modulation of said modulated emission signal and said input signal,

wherein said output signal is (a) indicative of a value of the fluorescence lifetime of said at least one fluorophore and (b) is independent of the intensity of the emitted light, and

wherein the comparing step comprises

dividing said emission signal into first and second signals;

filtering (a) said first signal by a high pass filter, followed by amplifying and splitting said first signal into a third signal and a fourth signal and (b) said second signal with a low pass filter;

amplifying and limiting said input signal and limiting said third signal, respectively, by passing said input signal through a signal amplifier set at a preset level and a limiting circuit, and by passing said third signal through a limiting circuit; then

mixing said third signal and said input signal, respectively, to generate a fifth signal; and

determining the ratio of said second and fourth signals as a ratio signal; and

comparing said ratio signal and said fifth signal in order to generate a value which is indicative of the fluorescence lifetime of said at least one fluorophore but which value is independent of the intensity of the emitted light.

8. A flow cytometer operative to measure a fluorescence lifetime of at least one fluorophore associated with a cell or a particle, comprising:

a flow chamber for directing a plurality of cells or particles past an observation point;

a light source modulated by a frequency of an input signal for irradiating, at said observation point, said cell or particle with modulated light having a wavelength capable of exciting said at least one fluorophore to produce emitted light;

a photodetector for detecting said emitted light and producing a corresponding emission signal; and

a phase/modulation detector for generating an output signal corresponding to a difference between the phase and/or modulation of said emission signal and said input signal, wherein said output signal is (a) indicative of a value of the fluorescence lifetime of said at least one fluorophore and (b) is independent of the intensity of the emitted light, and wherein said apparatus further comprises:

a first power splitter for dividing said input signal into first and second signals having the same amplitude and frequency;

a second power splitter for dividing said emission signal into third and fourth signals, said signals having an equal amplitude and identical frequency;

a first phase shifter for providing a predetermined phase shift to said first signal;

first and second mixers, said first mixer receiving said first signal of said first power splitter and said third signal from said second power splitter and generating a fifth signal, said second mixer receiving said second signal and said fourth signal and generating a sixth signal;

a ratio unit for receiving said fifth signal and said sixth signal from said first mixer and said second mixer, respectively, and generating the ratio of said fifth

signal and said sixth signal as said output signal.

12. A flow cytometer operative to measure a fluorescence lifetime of at least one fluorophore associated with a cell or a particle, comprising:

a flow chamber for directing a plurality of cells or particles past an observation point;

a light source modulated by a frequency of an input signal for irradiating, at said observation point, said cell or particle with modulated light having a wavelength capable of exciting said at least one fluorophore to produce emitted light;

a photodetector for detecting said emitted light and producing a corresponding emission signal; and

a phase/modulation detector for generating an output signal corresponding to a difference between the phase and/or modulation of said emission signal and said input signal, wherein said output signal is (a) indicative of a value of the fluorescence lifetime of said at least one fluorophore and (b) is independent of the intensity of the emitted light, and wherein said apparatus further comprises:

a first power splitter for dividing said emission signal into first and second signals, said signals having an equal amplitude and identical frequency;

a high pass filter for filtering said first signal;

a first amplifier and a second power splitter, respectively, for amplifying and splitting said first signal into a third signal and a fourth signal;

a first low pass filter for filtering said second signal;

a second amplifier and a first limiting circuit, respectively, for amplifying and limiting said input signal;

a second limiting circuit for limiting said third signal;

a mixer for mixing said third signal and said input signal, respectively, to generate a fifth signal;

a second low pass filter for filtering ,said fifth signal; and

a ratio unit for determining the ratio of said second and fourth signals as a ratio signal;

wherein said phase/modulation detector is fed said ratio signal and said fifth signal to determine said output signal.

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End of Result Set

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for claim 6

L19: Entry 3 of 3 File: USPT Apr 27, 1993

DOCUMENT-IDENTIFIER: US 5206143 A
TITLE: Method and reagents for performing subset analysis using quantitative differences in fluorescence intensity

Abstract Text (1):
Methods for distinguishing multiple subpopulations of biological particles in a single sample based upon quantitative differences in the fluorescence intensity attributable to one or two fluorochromes with which the biological particles are labelled. The method is used with flow cytometric particle counting techniques to count and sort and biological particles such as the formed elements of blood and other tissue cells. Also disclosed are reagents containing fluorochrome-conjugated antibodies used in the methods.

Detailed Description Text (52):
All analyses using a flow cytometer referred to in the following examples were performed using an EPICS 753 flow cytometer manufactured by Coulter Electronics of Hialeah, Fla. When using the fluorochromes phycoerythrin and/or fluorescein, 500 mw of light at an exciting wavelength of 488 nm was utilized. Also, a 488 nm dichroic mirror and 488 nm band pass for the right angle light scatter signal, a 515 nm interference filter and 515 nm long pass filter to block the excitation wavelength, a 560 nm dichroic mirror to split the fluorescein/phycoerythrin signal, a 590 nm longpass filter for the phycoerythrin signal, a 525 nm bandpass filter for the fluorescein signal, and a 1.5 OD filter for the forward angle light scatter signal were employed. When mononuclear cells were analyzed, gates were set around these cells using right angle light scatter and forward angle light scatter to remove any clumps or debris.

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End of Result Set

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L24: Entry 1 of 1 File: USPT Apr 28, 1998

DOCUMENT-IDENTIFIER: US 5744300 A
TITLE: Methods and reagents for the identification and regulation of senescence-related genes

Brief Summary Text (52):
As noted in Table 2 and in the scientific literature, a number of known genes are senescence-related genes. For instance, the activity of .beta.-galactosidase is elevated in senescent fibroblasts. Consequently, one can first conduct a primary screen of test compounds to determine whether that compound inhibits .beta.-galactosidase activity in senescent cells. In one embodiment of this screen, fibroblasts are grown to senescence, plated in 96 well plates, and incubated with a test compound. At the end of the incubation period, cells are analyzed for enzyme activity using a calorimetric assay based on the ability of the enzyme to cleave a colorless substrate into a colored reaction product. Compounds identified in this screen (see Example 14, below) as active compounds will then be tested in a secondary assay to determine that the active compounds are inhibiting the senescence-specific increase of activity of the enzyme and not merely inhibiting the enzyme itself. Other primary screens can be conducted using the senescence-related genes identified in Table 2, above, identified according to the methods of the invention, or known from the scientific literature. For instance, one could conduct a primary screen to identify compounds that have the capacity to induce the down-regulation of collagenase activity, an enzyme that is known to be elevated in senescent fibroblasts.

Detailed Description Text (255):
Samples that decrease staining in senescent cells are then tested for ability to decrease staining in young cells. In two preferred embodiments, young IMR90 cells are used at a PDL lower than 35, or young BJ cells are used at a PDL lower than 55. Other young cells, in the appropriate media, can also be used. The secondary screen is carried out in the same manner as the screen in senescent cells with two modifications. First, young cells, rather than senescent cells, are used. Second, the staining buffer is adjusted to pH 4 rather than pH 6. A decrease in .beta.-galactosidase staining in senescent but not young cells is interpreted as a reversal of the senescent phenotype.

WEST Search History

DATE: Tuesday, November 19, 2002

Set Name side by side	Query	Hit Count	Set Name result set
DB=JPAB,EPAB,DWPI; PLUR=NO; OP=ADJ			
L34	galactosidase near2 senescen$2	0	L34
L33	L32 near2 senescen$2	0	L33
L32	(b or beta) adj (gal or galactosidase)	1360	L32
L31	(sa) adj (b or beta) adj (gal or galactosidase)	0	L31
DB=PGPB; PLUR=NO; OP=ADJ			
L30	L29 and @ad<20000112	1	L30
L29	L25 or l27 or l28	12	L29
L28	galactosidase near2 senescen$2	2	L28
L27	L26 near2 senescen$2	11	L27
L26	(b or beta) adj (gal or galactosidase)	3232	L26
L25	(sa) adj (b or beta) adj (gal or galactosidase)	4	L25
DB=USPT; PLUR=NO; OP=ADJ			
L24	L23 or l22	1	L24
L23	galactosidase near2 senescen$2	1	L23
L22	L21 near2 senescen$2	1	L22
L21	(b or beta) adj (gal or galactosidase)	12521	L21
L20	(sa) adj (b or beta) adj (gal or galactosidase)	0	L20
L19	L18 and l8	3	L19
L18	L17[ti,ab]	1033	L18
L17	L2 or l3 or l4	20233	L17
L16	L14 and l3	0	L16
L15	L14 and l4	0	L15
L14	4771468[pn]	1	L14
L13	L12 or l11 or l10	5	L13
L12	L8 same l2	3	L12
L11	L8 same l3	1	L11
L10	L8 same l4	1	L10
L9	L8 with l4	0	L9
L8	split$4 near2 signal	7850	L8
L7	L5 same l4	1	L7
L6	L5 with l4	0	L6

L5	split$4 near signal	4911	L5
L4	fluoresce$3 adj2 microsco$3	4896	L4
L3	flow adj2 cytometr$2	5787	L3
L2	image near analy$4	11438	L2
L1	image adj analy$4	9599	L1

END OF SEARCH HISTORY